

## SEQUENCE LISTING

<110> University of Wales, Bangor

Trwyn Ltd

<120> Improvements In and Relating to Biosensors

<130> BA/SLH/Y1861

<160> 9

<170> PatentIn version 3.1

<210> 1

<211> 654

<212> DNA

<213> Escherichia coli K12

<400> 1

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accaactccc agccgtggca ttttattggt gccagcacgg aagaaggtaa agcgcgtggt     180
gccaaatccg ctgccggtaa ttacgtgttc aacgagcgta aaatgcttga tgcctcgcac     240
gtcgtggtgt tctgtgcaaa aaccgcgatg gacgatgtct ggctgaagct ggttggtgac     300
caggaagatg ccgatggccg ctttgccacg ccggaagcga aagccgcgaa cgataaaggt     360
cgcaagttct tcgctgatat gcaccgtaaa gatctgcatg atgatgcaga gtggatggca     420
aaacagggttt atctcaacgt cggtaacttc ctgctcggcg tggcggctct gggctctggac     480
gcggtaccca tcgaagggttt tgacgccgcc atcctcgatg cagaatttgg tctgaaagag     540
aaaggctaca ccagtctggt ggttggtccg gtaggtcatc acagcgttga agattttaac     600
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<210> 2

<211> 826

<212> DNA

<213> *Pseudomonas putida* JLR11

<400> 2

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agcgatcttc ctgtggatga gcagatgctg agctggggcga tcgcggcggc ccagtcagcc	180
tcgacttcct cgaacctgca agcttggagc gtgctcgccg tgcgggatcg cgagcgtctc	240
gcgaggcttg cccgactgtc cggtaaccag cgccatgtcg agcaggcacc gctgttcctg	300
gtctggctcg tggactggtc acgcctacgc cgactagcca gaacccttca ggcaccgact	360
gcaggtatcg actatctaga aagctacacc gtcggtgttg tagatgcagc tctggccgct	420
cagaacgccg cactagcttt cgaggcccaa ggactgggaa tcgtttacat cggcggaatg	480
cgcaaccacc cggaagcgat gtccgaggag cttggcctgc caaacgacac tttcgctgta	540
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gcgcaatcag tgggtgcttca ccgtgagcgc tatgaggcca ccgaggcaga ggcgggttca	660
gttgctgcct atgaccgaag gatgagcgcac ttccaacatc gtcaacaacg cgaaaaccgt	720
tcctgggtcca gccaggccgt ggaacgtgta aaaggagcgg attcactgag cggaagacac	780
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<212> DNA

<213> *Escherichia coli* K12 nfnB in pET-28(a)(+); pMKS2

<220>

<221> CDS

<222> (88)..(858)

<223> Coding sequence for nfnB gene

<220>

<221> misc\_feature

<222> (250)..(267)

<223> Cys tags

<220>

<221> misc\_feature

<222> (160)..(177)

<223> His tags

<220>

<221> misc\_feature

<222> (268)..(285)

<223> primer

<220>

<221> misc\_feature

<222> (996)..(1010)

<223> primer

<400> 3

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Met Gly Ser Ser His His His His His  
1 5

cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gct agc atg act 162  
His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr  
10 15 20 25

ggt gga cag caa atg ggt cgc gga tcc tgt tgc tgt tgc tgt tgc gat 210  
Gly Gly Gln Gln Met Gly Arg Gly Ser Cys Cys Cys Cys Cys Cys Asp  
30 35 40

atc att tct gtc gcc tta aag cgt cat tcc act aag gca ttt gat gcc 258  
Ile Ile Ser Val Ala Leu Lys Arg His Ser Thr Lys Ala Phe Asp Ala  
45 50 55

agc aaa aaa ctt acc ccg gaa cag gcc gag cag atc aaa acg cta ctg 306  
Ser Lys Lys Leu Thr Pro Glu Gln Ala Glu Gln Ile Lys Thr Leu Leu  
60 65 70

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caa tac agc cca tcc agc acc aac tcc cag ccg tgg cat ttt att gtt	354
Gln Tyr Ser Pro Ser Ser Thr Asn Ser Gln Pro Trp His Phe Ile Val	
75 80 85	
gcc agc acg gaa gaa ggt aaa gcg cgt gtt gcc aaa tcc gct gcc ggt	402
Ala Ser Thr Glu Glu Gly Lys Ala Arg Val Ala Lys Ser Ala Ala Gly	
90 95 100 105	
aat tac gtg ttc aac gag cgt aaa atg ctt gat gcc tcg cac gtc gtg	450
Asn Tyr Val Phe Asn Glu Arg Lys Met Leu Asp Ala Ser His Val Val	
110 115 120	
gtg ttc tgt gca aaa acc gcg atg gac gat gtc tgg ctg aag ctg gtt	498
Val Phe Cys Ala Lys Thr Ala Met Asp Asp Val Trp Leu Lys Leu Val	
125 130 135	
gtt gac cag gaa gat gcc gat ggc cgc ttt gcc acg ccg gaa gcg aaa	546
Val Asp Gln Glu Asp Ala Asp Gly Arg Phe Ala Thr Pro Glu Ala Lys	
140 145 150	
gcc gcg aac gat aaa ggt cgc aag ttc ttc gct gat atg cac cgt aaa	594
Ala Ala Asn Asp Lys Gly Arg Lys Phe Phe Ala Asp Met His Arg Lys	
155 160 165	
gat ctg cat gat gat gca gag tgg atg gca aaa cag gtt tat ctc aac	642
Asp Leu His Asp Asp Ala Glu Trp Met Ala Lys Gln Val Tyr Leu Asn	
170 175 180 185	
gtc ggt aac ttc ctg ctc ggc gtg gcg gct ctg ggt ctg gac gcg gta	690
Val Gly Asn Phe Leu Leu Gly Val Ala Ala Leu Gly Leu Asp Ala Val	
190 195 200	
ccc atc gaa ggt ttt gac gcc gcc atc ctc gat gca gaa ttt ggt ctg	738
Pro Ile Glu Gly Phe Asp Ala Ala Ile Leu Asp Ala Glu Phe Gly Leu	
205 210 215	
aaa gag aaa ggc tac acc agt ctg gtg gtt gtt ccg gta ggt cat cac	786
Lys Glu Lys Gly Tyr Thr Ser Leu Val Val Val Pro Val Gly His His	
220 225 230	
agc gtt gaa gat ttt aac gct acg ctg ccg aaa tct cgt ctg ccg caa	834
Ser Val Glu Asp Phe Asn Ala Thr Leu Pro Lys Ser Arg Leu Pro Gln	
235 240 245	
aac atc acc tta acc gaa gtg taa ttctctcttg ccgggcatct gcccggtat	888
Asn Ile Thr Leu Thr Glu Val	
250 255	
ttcctctcag attctcctga tttgcataac cctgtttcag caagcttcgt catcataggc	948
tgctgttgaa gcttgcggcc gactcgagc accaccacca ccaccactga gatccggctg	1008
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<210> 4

<211> 256

<212> PRT

<213> Escherichia coli K12 nfnB in pET-28(a)(+); pMKS2

<220>

<221> misc\_feature

<222> (250)..(267)

<223> Cys tags

<220>

<221> misc\_feature

<222> (160)..(177)

<223> His tags

<220>

<221> misc\_feature

<222> (268)..(285)

<223> primer

<220>

<221> misc\_feature

<222> (996)..(1010)

<223> primer

<400> 4

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Arg	Gly	Ser	His	Met	Ala	Ser	Met	Thr	Gly	Gly	Gln	Gln	Met	Gly	Arg
			20					25					30		

Gly	Ser	Cys	Cys	Cys	Cys	Cys	Cys	Asp	Ile	Ile	Ser	Val	Ala	Leu	Lys
		35					40					45			

Arg	His	Ser	Thr	Lys	Ala	Phe	Asp	Ala	Ser	Lys	Lys	Leu	Thr	Pro	Glu
	50					55					60				

Gln	Ala	Glu	Gln	Ile	Lys	Thr	Leu	Leu	Gln	Tyr	Ser	Pro	Ser	Ser	Thr
65					70				75						80

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Asn Ser Gln Pro Trp His Phe Ile Val Ala Ser Thr Glu Glu Gly Lys  
85 90 95

Ala Arg Val Ala Lys Ser Ala Ala Gly Asn Tyr Val Phe Asn Glu Arg  
100 105 110

Lys Met Leu Asp Ala Ser His Val Val Val Phe Cys Ala Lys Thr Ala  
115 120 125

Met Asp Asp Val Trp Leu Lys Leu Val Val Asp Gln Glu Asp Ala Asp  
130 135 140

Gly Arg Phe Ala Thr Pro Glu Ala Lys Ala Ala Asn Asp Lys Gly Arg  
145 150 155 160

Lys Phe Phe Ala Asp Met His Arg Lys Asp Leu His Asp Asp Ala Glu  
165 170 175

Trp Met Ala Lys Gln Val Tyr Leu Asn Val Gly Asn Phe Leu Leu Gly  
180 185 190

Val Ala Ala Leu Gly Leu Asp Ala Val Pro Ile Glu Gly Phe Asp Ala  
195 200 205

Ala Ile Leu Asp Ala Glu Phe Gly Leu Lys Glu Lys Gly Tyr Thr Ser  
210 215 220

Leu Val Val Val Pro Val Gly His His Ser Val Glu Asp Phe Asn Ala  
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Thr Leu Pro Lys Ser Arg Leu Pro Gln Asn Ile Thr Leu Thr Glu Val  
245 250 255

<210> 5

<211> 1221

<212> DNA

<213> Pseudomonas putida JLR11 prnB in pET-28(a)(+) ; pKMS6

<220>

<221> CDS

<222> (88) .. (1029)

<223>

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<222> (190) .. (225)

<223> primer

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<222> (190) .. (207)

<223> cys tag

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<222> (936) .. (956)

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tgtttaactt taagaaggag atataacc atg ggc agc agc cat cat cat cat cat 114  
Met Gly Ser Ser His His His His His  
1 5

cac agc agc ggc ctg gtg ccg cgc ggc agc cat atg gct agc atg act 162  
His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr  
10 15 20 25

ggt gga cag caa atg ggt cgc gga tcc tgt tgc tgt tgc tgt tgc agc 210  
Gly Gly Gln Gln Met Gly Arg Gly Ser Cys Cys Cys Cys Cys Cys Ser  
30 35 40

ctt caa gac gaa gca ctc aaa gcc tgg caa gcc cgt tat ggc gag cca 258  
Leu Gln Asp Glu Ala Leu Lys Ala Trp Gln Ala Arg Tyr Gly Glu Pro  
45 50 55

gct aac tta cct gct gcc gac acc gtg atc gcg cag atg ttg cag cat 306  
Ala Asn Leu Pro Ala Ala Asp Thr Val Ile Ala Gln Met Leu Gln His

60	65	70	
cga tca gta cgt gcc tac agc gat ctt cct gtg gat gag cag atg ctg Arg Ser Val Arg Ala Tyr Ser Asp Leu Pro Val Asp Glu Gln Met Leu 75 80 85			354
agc tgg gcg atc gcg gcg gcc cag tca gcc tcg act tcc tcg aac ctg Ser Trp Ala Ile Ala Ala Ala Gln Ser Ala Ser Thr Ser Ser Asn Leu 90 95 100 105			402
caa gct tgg agc gtg ctc gcc gtg cgg gat cgc gag cgt ctc gcg agg Gln Ala Trp Ser Val Leu Ala Val Arg Asp Arg Glu Arg Leu Ala Arg 110 115 120			450
ctt gcc cga ctg tcc ggt aac cag cgc cat gtc gag cag gca ccg ctg Leu Ala Arg Leu Ser Gly Asn Gln Arg His Val Glu Gln Ala Pro Leu 125 130 135			498
ttc ctg gtc tgg ctc gtg gac tgg tca cgc cta cgc cga cta gcc aga Phe Leu Val Trp Leu Val Asp Trp Ser Arg Leu Arg Arg Leu Ala Arg 140 145 150			546
acc ctt cag gca ccg act gca ggt atc gac tat tta gaa agc tac acc Thr Leu Gln Ala Pro Thr Ala Gly Ile Asp Tyr Leu Glu Ser Tyr Thr 155 160 165			594
gtc ggt gtt gta gat gca gct ctg gcc gct cag aac gcc gca cta gct Val Gly Val Val Asp Ala Ala Leu Ala Ala Gln Asn Ala Ala Leu Ala 170 175 180 185			642
ttc gag gcc caa gga ctg gga atc gtt tac atc ggc gga atg cgc aac Phe Glu Ala Gln Gly Leu Gly Ile Val Tyr Ile Gly Gly Met Arg Asn 190 195 200			690
cac ccg gaa gcg atg tcc gag gag ctt ggc ctg cca aac gac act ttc His Pro Glu Ala Met Ser Glu Glu Leu Gly Leu Pro Asn Asp Thr Phe 205 210 215			738
gct gta ttt ggc atg tgc gtc ggt cat ccc gat ccg gca cag ccc gcc Ala Val Phe Gly Met Cys Val Gly His Pro Asp Pro Ala Gln Pro Ala 220 225 230			786
gag atc aag cca cgc ctg gcg caa tca gtg gtg ctt cac cgt gag cgc Glu Ile Lys Pro Arg Leu Ala Gln Ser Val Val Leu His Arg Glu Arg 235 240 245			834
tat gag gcc acc gag gca gag gcg gtt tca gtt gct gcc tat gac cga Tyr Glu Ala Thr Glu Ala Glu Ala Val Ser Val Ala Ala Tyr Asp Arg 250 255 260 265			882
agg atg agc gac ttc caa cat cgt caa caa cgc gaa aac cgt tcc tgg Arg Met Ser Asp Phe Gln His Arg Gln Gln Arg Glu Asn Arg Ser Trp 270 275 280			930
tcc agc cag gcc gtg gaa cgt gta aaa gga gcg gat tca ctg agc gga Ser Ser Gln Ala Val Glu Arg Val Lys Gly Ala Asp Ser Leu Ser Gly 285 290 295			978
aga cac cgc ttg cga gat gca tta aac acc cta ggt ttc ggc ctg cgc			1026



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Arg His Arg Leu Arg Asp Ala Leu Asn Thr Leu Gly Phe Gly Leu Arg  
300 305 310

tga gatagtgaga tatcccatgc ctattcccgc cgccctgaac cggagcacta 1079  
atacctggca actttgcttg agctccgtcg acaagcttgc ggccgcactc gagcaccacc 1139  
accaccacca ctgagatccg gctgctaaca aagcccgaag ggaagctgag ttggctgctg 1199  
ccaccgctga gcaataacta gc 1221

<210> 6

<211> 313

<212> PRT

<213> Pseudomonas putida JLR11 prnB in pET-28(a)(+) ; pKMS6

<220>

<221> misc\_feature

<222> (190)..(225)

<223> primer

<220>

<221> misc\_feature

<222> (190)..(207)

<223> cys tag

<220>

<221> misc\_feature

<222> (936)..(956)

<223> primer

<400> 6

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Arg Gly Ser His Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg  
20 25 30

Gly Ser Cys Cys Cys Cys Cys Ser Leu Gln Asp Glu Ala Leu Lys  
35 40 45

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Ala	Trp	Gln	Ala	Arg	Tyr	Gly	Glu	Pro	Ala	Asn	Leu	Pro	Ala	Ala	Asp
50						55					60				
Thr	Val	Ile	Ala	Gln	Met	Leu	Gln	His	Arg	Ser	Val	Arg	Ala	Tyr	Ser
65					70					75					80
Asp	Leu	Pro	Val	Asp	Glu	Gln	Met	Leu	Ser	Trp	Ala	Ile	Ala	Ala	Ala
				85					90					95	
Gln	Ser	Ala	Ser	Thr	Ser	Ser	Asn	Leu	Gln	Ala	Trp	Ser	Val	Leu	Ala
			100					105					110		
Val	Arg	Asp	Arg	Glu	Arg	Leu	Ala	Arg	Leu	Ala	Arg	Leu	Ser	Gly	Asn
		115					120					125			
Gln	Arg	His	Val	Glu	Gln	Ala	Pro	Leu	Phe	Leu	Val	Trp	Leu	Val	Asp
		130				135					140				
Trp	Ser	Arg	Leu	Arg	Arg	Leu	Ala	Arg	Thr	Leu	Gln	Ala	Pro	Thr	Ala
145					150					155					160
Gly	Ile	Asp	Tyr	Leu	Glu	Ser	Tyr	Thr	Val	Gly	Val	Val	Asp	Ala	Ala
				165					170					175	
Leu	Ala	Ala	Gln	Asn	Ala	Ala	Leu	Ala	Phe	Glu	Ala	Gln	Gly	Leu	Gly
			180					185					190		
Ile	Val	Tyr	Ile	Gly	Gly	Met	Arg	Asn	His	Pro	Glu	Ala	Met	Ser	Glu
		195					200					205			
Glu	Leu	Gly	Leu	Pro	Asn	Asp	Thr	Phe	Ala	Val	Phe	Gly	Met	Cys	Val
	210					215					220				
Gly	His	Pro	Asp	Pro	Ala	Gln	Pro	Ala	Glu	Ile	Lys	Pro	Arg	Leu	Ala
225					230					235					240
Gln	Ser	Val	Val	Leu	His	Arg	Glu	Arg	Tyr	Glu	Ala	Thr	Glu	Ala	Glu
				245					250					255	
Ala	Val	Ser	Val	Ala	Ala	Tyr	Asp	Arg	Arg	Met	Ser	Asp	Phe	Gln	His
			260					265					270		
Arg	Gln	Gln	Arg	Glu	Asn	Arg	Ser	Trp	Ser	Ser	Gln	Ala	Val	Glu	Arg
		275					280					285			

Val Lys Gly Ala Asp Ser Leu Ser Gly Arg His Arg Leu Arg Asp Ala  
 290 295 300

Leu Asn Thr Leu Gly Phe Gly Leu Arg  
 305 310

<210> 7

<211> 24

<212> DNA

<213> Escherichia coli

<400> 7  
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24

<210> 8

<211> 27

<212> DNA

<213> Escherichia coli

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27

<210> 9

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<213> Artificial Sequence

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<223> Primer consisting of nfnB gene primer shown in SEQ ID4 with an additional 6 cysteine codons

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42